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Investigation of the meiotic behavior in some *Echinops* L. (Asteraceae) species from Iran

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Abstract

Investigation of the meiotic behavior in some *Echinops* L. (Asteraceae) species from Iran.— *Echinops* L., is a genus of Asteraceae and contains *ca*. 76 species in Iran. The current investigation was performed on six species and nine populations including *E. cephalotus*, *E. chorassanicus*, *E. elebursessis*, *E. leiopolycerus*, *E. ritroides* and *E. robustus*. Chiasma frequency and distribution, chromosomal association and segregation were analyzed for meiotic characters. Meiotic irregularities, unreduced chromosomes and other related abnormalities were observed in the studied species. Chromosome stickiness, laggard chromosomes as well as frequent tripolar and multipolar cell formation due to anaphase I and II failure were observed.

Key words: *Echinops*; meiotic irregularities and abnormalities; unreduced pollen grains.

Resumen

Investigación sobre el comportamiento meiótico en varias especies de *Echinops* L. (*Asteraceae*) de Irán.— *Echinops* L., es un género de *Asteraceae* y contiene *ca*. 76 especies en Irán. La investigación actual se basa en seis especies y nueve poblaciones, incluidas *E. cephalotus*, *E. chorassanicus*, *E. elebursessis*, *E. leiopolycerus*, *E. ritroides* y *E. robustus*. La frecuencia y distribución de quiasmas, la asociación cromosómica y la segregación han sido analizadas para caracteres meióticos. En las especies estudiadas se han observado irregularidades meióticas, cromosomas no reducidos y otras anomalías relacionadas. Se observan también adherencia cromosómica, cromosomas rezagados, así como frecuentes formaciones de células tripolares y multipolares debido a fallos en la anafase I y II.

Palabras clave: *Echinops*; granos de polen no reducidos; irregularidades y anomalías meióticas.

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INTRODUCTION

Echinops L., belongs to family Asteraceae Bercht. & J. Presl, subfamily Carduoideae Cass. ex Sweet., tribe Cardueae Cass. (Cynareae Lam. & DC., Echinopsinae) (Susanna *et al.*, 2006). Characters like compound inflorescence and single-flowered capitula congested into secondary inflorescences appearing as spherical or oval heads are the exceptional and diagnostic characteristics in this tribe. The taxa of this genus are mostly perennial with few annuals (Petit, 1997). *Echinops* encompasses approximately 120 species (Bobrov, 1997; Susanna & Garcia-Jacas, 2007) occurring in the north and tropical Africa, the Mediterranean basin, and temperate habitats in Eurasia up to central Asia and north-eastern China. The Caucasus and the Middle East are the two regions where most species grow (Bobrov, 1962; Jäger, 1987; Meusel & Jäger, 1992; Susanna & Garcia- Jacas, 2009; Sánchez-Jiménez *et al.*, 2010). For the *Flora Iranica* area almost 76 species were recorded in five sections (Rechinger, 1979) and subsequently Mozaffarian (2002, 2006, 2008*a*, *b*) and Mozaffarian & Ghahreman (2002*a*, *b*) added 19 new species of this genus to the treaty. About 50 species of *Echinops* are endemic to Iran. Hence, Iran is one of the most important diversification centers of this genus in the world (Montazerolghaem *et al.*, 2017).

In Asteraceae, the chromosome number ranges from n = 2 to n = 114 (Funk *et al.*, 2005) and high ploidy levels are present. Cytological investigations of *Echinops* have been mostly focused on chromosome number and karyotype analysis (Strid & Franzen, 1981; Moore, 1982; Goldblatt & Johnson, 1983; Ghaffari, 1999; Sheidai *et al.*, 2000; Garnatje *et al.*, 2004*a*, *b*; Sánchez-Jiménez *et al.*, 2012). Chromosome numbers in *Echinops* range from 2n = 26 (*E. gmelini* Turcz) (Sánchez-Jiménez *et al.*, 2009) to 2n = 36 (*E. transcaucasicus* Iljin). Most of the reported chromosome numbers are 2n = 28, 30, 32, 34 and 36 (Ghaffari, 1999; Sheidai *et al.*, 2000; Alijanpoor *et al.*, 2019*a*, *b*).

The main aim of this survey is to study the meiotic behavior and the pollen grain morphology in some *Echinops* species.

MATERIAL AND METHODS

Plant material

Floral buds of nine populations from six *Echinops* species were collected from Tehran and Alborz slope regions (April to August 2012) in different natural habitats. Voucher specimens are deposited in the herbariums IRAN (Iranian Research Institute of Plant Protection) and HSBU (Herbarium of Shahid Beheshti University), details are presented in Table 1. Species were identified based on *Flora Iranica* (Rechinger, 1979) and compared with the specimens deposited at these herbaria.

Meiotic studies

For the meiotic analysis, young flower buds, collected from at least 10 plants, were randomly selected. After being fixed in a mixture of ethanol and glacial acetic acid in a volume ratio (3:1) for 24 h, these were subsequently transferred to 70% ethanol for 24 h and stored at 5°C until use. Meiocytes were prepared by squashing anthers and stained with acetocarmine (1%). Chromosome numbers were determined for a minimum of 100 metaphase/diakinesis pollen mother cells (PMCs), and 500 anaphase and telophase cells were analyzed for data collection from freshly prepared slides (Sheidai *et al.*, 1999, 2002). Bright field images were obtained using an Olympus BX-60 microscope (Olympus, Tokyo, Japan).

Pollen grain analysis

Pollen fertility and size frequencies were analyzed through stain ability tests using 2% acetocarmine: 50% glycerin (1:1) for about 30 min (Sheidai *et al.*, 2010). Up to 1000 pollen grains were examined. Round complete pollen grains with stained nuclei were taken as apparently fertile while shriveled and unstained pollens were considered as infertile.

Statistical analyses

Student's *t*-test analysis for the purpose of significant difference in mean total and relative chiasma frequency was performed. One-way analysis of variance (ANOVA) was applied with Duncan test to decide the contrasts between different *Echinops* species. The Pearson coefficient of correlation was applied to address the relationship between pollen fertility, anaphase, and telophase laggard chromosomes with extra chromosomes. In order to group the nine populations showing similar meiotic behavior, WARD and different methods of cluster analyses, including single linkage, UPGMA as well

Table 1. *Echinops* species studied in the present work.

Species	Locality	2*n*	Voucher no.	Altitude (m)	Geographic coordinates
E. cephalotes DC.	Teheran, Parchin, Road, Khojir	32	HSBU2019902	1470	35° 42’ 40” N 51° 38’ 14” E
E. cephalotes DC.	Teheran, Qarchak	32	HSBU2019904	1022	35° 23’ 59” N 51° 35’ 29” E
E. chorassanicus Bunge	Teheran, Damavand Road, Kamard	32	HSBU2019908	1610	35° 44’ 53” N 51° 44’ 10” E
E. chorassanicus Bunge	Haraz, Polour	32	HSBU2019907	2163	35° 50’ 6” N 52° 3’ 32” E
E. elbursensis Rech.f.	Haraz Road, Emamzadeh Hashem	32	IRAN67131	2616	35° 57’ 28” N 52° 18’ 54” E
E. leiopolycerus Bornm.	Haraz Road, Abali	32	HSBU2019905	2332	35° 45’ 37” N 51° 57’ 46” E
E. leiopolycerus Bornm.	Teheran, Damavand Road, Kamard	32	IRAN67126	1610	35° 44’ 33” N 51° 44’ 13” E
E. ritroides Bunge	Haraz Road, Abali	34	HSBU2019909	2332	35° 45’ 41” N 51° 57’ 45” E
E. robustus Bunge	Qom Road, Nalbandad	32	HSBU2019900	1154	34° 44’ 11” N 50° 47’ 7” E

as ordination based on principal components analysis (PCA) were utilized (Sheidai *et al.*, 2002). For this analysis, at least 50 larger pollen grains and 50 smaller pollen grains were randomly measured. For the statistical analyses SPSS v16, and PAST v4.06b were used.

RESULTS

Meiotic abnormalities

The genus *Echinops* was studied cytogenetically, with chromosome counts $2n = 2x = 32$ and 34. In *E. ritroides* (Haraz), many unreduced pollen grains (2*n*) were observed. Laggard chromosomes, anaphase and telophase II failure, chromosomes stickiness, abnormal tripolar, micronucleus, and quadrivalent formation were also observed. In *E. eleborensis* (Haraz), abnormal metaphase I/II, laggard, metaphase II failure, multipolar (tetrapolar and triad) were detected. In *E. cephalotes*, (Qarchak population), anaphase I/II failure, telophase failure, micronucleus, syncyte, multipolar, and normal pollen grains were seen, however, compared to the population of Khojir two meiotic characters such as anaphase II laggards and metaphase I stickiness were not observed. In *E. chorassanicus* (Polour population), anaphase I/II, metaphase I stickiness, laggard, and tripolar abnormalities were identified. While in the *E. chorassanicus* (Kamard) population anaphase I laggards and metaphase I stickiness were not observed. In *E. leiopolycerus* (Kamard population), metaphase I stickiness, telophase II failure, with variation in shape and size of unreduced pollen grains, was created. In *E. leiopolycerus* (Abali population), metaphase I/II anaphase II failure, syncyte, micronucleus, triad, tetrapolar, unreduced pollen grain with variation in shape and size revealed. Finally, in *E. robustus* (Qom) species all meiotic characters were gained except anaphase II laggard percentage (Table 2). The meiotic traits investigated in this study include terminal chiasmata/bivalent, intercalary chiasmata/bivalent, total chiasmata/bivalent, ring bivalent/cell, rod bivalent/cell, size of normal pollen grains (μm), and unreduced pollen grains (μm). Also, chromosomal abnormalities related to the behavior in metaphase I/II telophase I/II, laggard, anaphase II failure, telophase II failure, stickiness, micronucleus, diffuse, meiotic irregularities like tripolar and multipolar were studied [Fig. 3 (1–27)]. Duncan’s test presented a significant difference $p < 0.05$ in meiotic characteristics such as mean number of terminal chiasmata/bivalent (TXN), mean

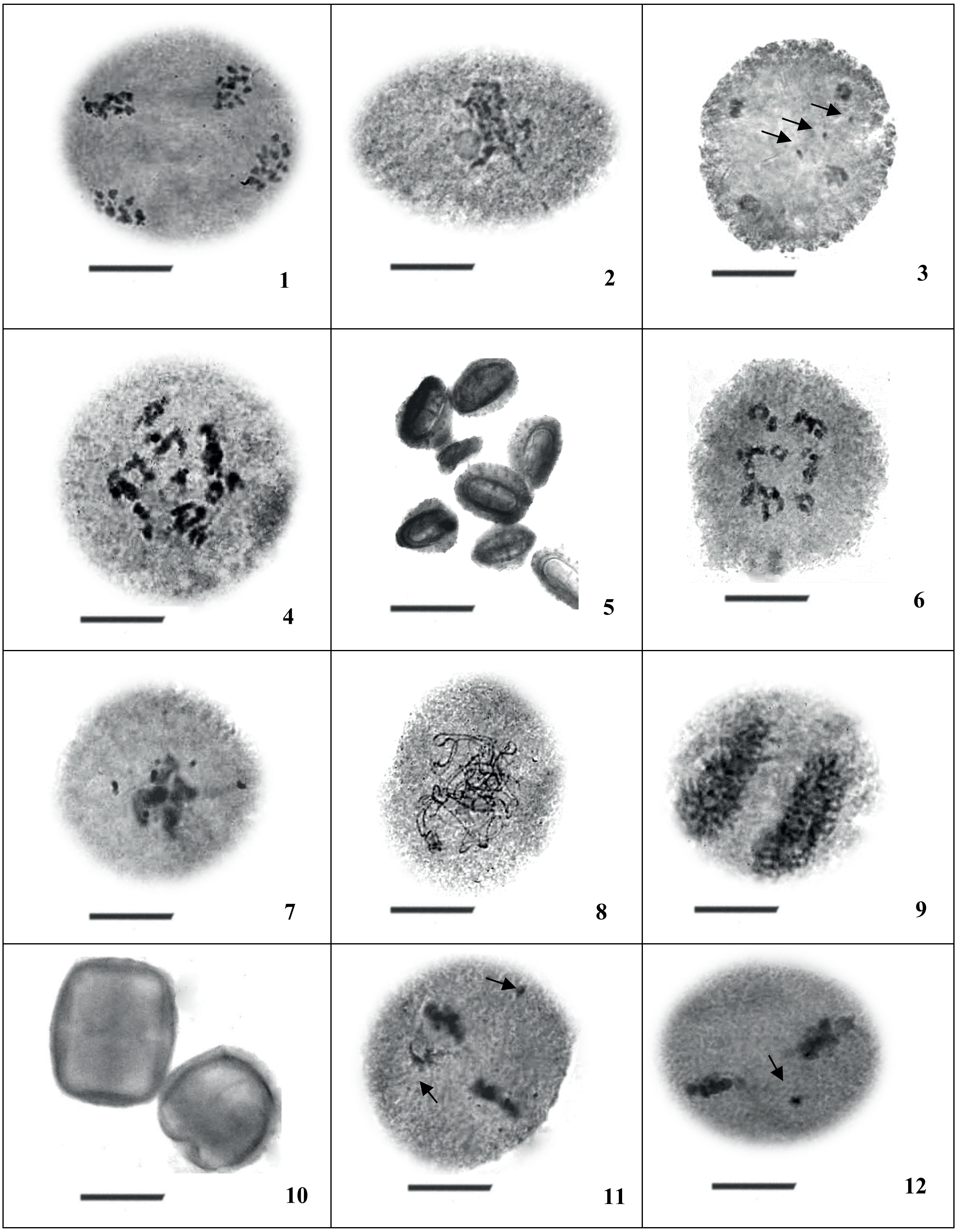

Figure 3. (1–27) *Echinops cephalotes* (Khojir): (1), anaphase I; (2), syncyte; Qarchak population: (3), micronuclei formation (arrow); (4), diakinesis; (5), variation in shape and size of pollen grain (*n*, *2n*, normal & infertile); *E. chorassanicus* (Polour): (6), diakinesis; (7), metaphase I stickiness; (8), leptotene; Kamard population: (9), telophase II (abnormal); (10), pollen grain (*n*, *2n*); *E. leiopolycerus* (Kamard): (11), laggard (arrows); (12), metaphase II and laggard (arrow); (13), diakinesis; Abali population: (14), anaphase II stickiness; (15), pollen grain (*n*, *2n*); *E. elbersensis* (E-hashem): (16), diakinesis; (17), irregular tetrapolar; (18), failure anaphase I stickiness; (19), irregular tetrapolar; (20), pollen grain variation in shape (oval and triangular); *E. robustus* (Qom): (21), diakinesis; (22), failure anaphase I; (23), multipolar; (24), pollen grain (*n*, *2n*); *E. ritroides* (Abali): (25), diakinesis; (26), pollen grain (*n*, *2n*); (27), tetrapolar. Scale bar = 10 µm.

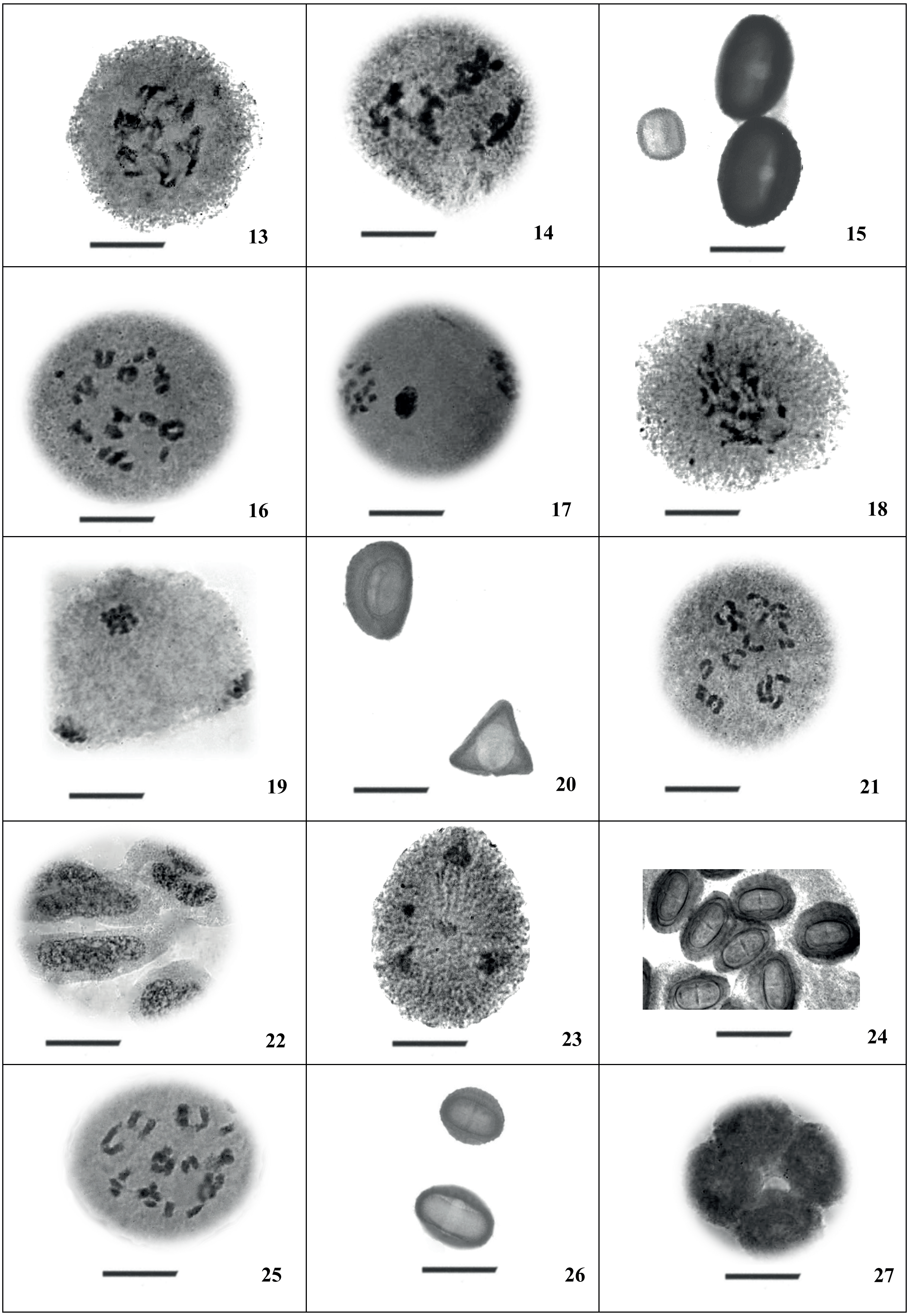
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Table 2. Frequency of chiasmata and size of pollen grains in *Echinops* species studied. TXN: mean number of terminal chiasmata/bivalent; IXN: mean number of intercalary chiasmata/bivalent; TOXN: mean number of total chiasmata/bivalent; IX: mean number of intercalary chiasmata; TX: mean number of terminal chiasmata; TOX: mean number of total chiasmata; RB: mean number of ring bivalents; RD: mean number of rod bivalents; A1L: anaphase I laggards percentage; A2L: anaphase II laggards percentage; MST: metaphase I stickiness percentage; NP: size of normal pollen grain (μm); UP: size of unreduced pollen grain (μm); SS: size of small pollen grain (μm).

Species	TXN	IXN	TOXN	IX	TX	TOX	RB	RD	A1L	A2L	MST	NP	UP	SS
E. cephalotes (Khojir)	13.77	5.14	19.22	4.40	16.44	26.01	10.5	0.05	0.4	–	–	56.56	60.23	45.6
*E. cephalotes (*Qarchak)	9.5	12.25	20.62	12.25	30.62	6	17.4	0.8	–	0.1	–	61.70	50.33	47.3
E. chorassanicus (Polour)	13.2	11.5	24.8	11.5	20.51	22.88	8.90	1.36	0.1	0.1	0.2	68.44	61.33	55.34
E. chorassanicus (Kamard)	18.60	11.7	22.41	5.11	28.24	19.5	1.90	21.40	–	0.3	–	67.59	62.31	58.87
E. ritroides (Abali)	16.30	9	25.20	9	27.25	25.41	18.23	0.70	0.7	0.6	0.4	53.89	62.57	44.44
E. elbursensis (E-hashem)	9.63	9.06	18.19	6.5	31.18	4.45	18.21	6.95	–	–	0.1	57.04	–	–
E. robustus (Qom)	12.57	12.43	25.29	3.12	31.25	22.30	2	25.44	0.1	–	0.1	52.73	61.4	48.69
E. leiopolycerus (Kamard)	12.23	11.46	23.54	10.11	25.23	13.99	14.5	0.24	–	–	0.2	65.79	95.8	50.16
E. leiopolycerus (Abali)	12.70	11.42	23.10	10.61	16.54	12.74	11.4	0.77	–	–	0.1	82.96	107.23	72.72

number of intercalary chiasmata/bivalent (IXN), mean number of total chiasmata/bivalent (TOXN) and size of normal pollen grain (NP) among the species and populations studied (Table 3).

Ploidy level and chiasma frequency

Among *Echinops* species, the highest mean number of terminal chiasmata and total chiasmata (31.25, 26.01) occurred in *E. robustus* (Qom population) and *E. cephalotes* (Khojir population), respectively, while the lowest mean number of the total, intercalary and terminal chiasmata was obtained in *E. elbursensis* (Emamzadeh Hashem), *E. robustus* (Qom) and *E. cephalotes* (Khojir) (4.45, 16.44 and 3.12, respectively) (Table 2). This study showed that mostly rod and ring shapes of diakinesis chromosomes process in meiosis-I metaphase. Additionally, pollen fertility showed >0.90%. The correlation test showed no significant correlation between relative total, terminal and intercalary chiasmata as well as ring and rod bivalents with change in chromosome number. Cluster analysis UPGMA and ordination according to principal components analysis (PCA) of meiotic characters generated similar outcomes (Figs. 1 and 2).

Unreduced pollen grain formation

Pollen grains sizes of all six species were measured. The large pollen grains (unreduced pollen grains) were obtained in *E. leiopolycerus* (Abali population) (107.23 μm) and smaller pollen grains (normal) were from *E. ritroides* (Abali population) (44.44 μm) (Table 2). The pollen fertility of all species was more than 90%. It should be noted that a variety of shapes (oval and triangular) and size of pollen grains were also observed in studied species. Student's *t*-test analysis disclosed a significant difference ($p < 0.05$) for the size between the larger and smaller size pollen grains in all species of *Echinops* [Fig. 3 (15, 20, 26)].

DISCUSSION

In the previous work of Sheidai *et al*. (2000) the chromosome number of *E. leiopolyceras* $2n = 30$ was reported. Similarly, for *E. ritrodes*, the

Table 3. Mean values of the different meiotic configurations and chromosome associations in *Echinops* species and populations. TXN: mean number of terminal chiasmata/bivalent; IXN: mean number of intercalary chiasmata/bivalent; TOXN: mean number of total chiasmata/bivalent; NP: size of normal pollen grain (µm). The mean values followed by the same letter(s) in a column are not significantly different by Duncan's test at ($p < 0.05$)

Species	TXN	IXN	TOXN	NP
E. cephalotes (Khojir)	13.77 ± 0.007^{f}	5.13 ± 0.007^{a}	19.21 ± 0.007^{b}	56.56 ± 0.007^{c}
E. cephalotes (Qarchak)	9.55 ± 0.07^{a}	12.25 ± 0.007^{f}	20.61 ± 0.007^{c}	61.70 ± 0.007^{e}
E. chorassanicus (Kamard)	18.60 ± 0.007^{h}	11.70 ± 0.007^{e}	22.41 ± 0.007^{d}	67.58 ± 0.007^{g}
E. chorassanicus (Polour)	13.15 ± 0.07^{e}	11.49 ± 0.007^{d}	24.80 ± 0.007^{g}	68.44 ± 0.007^{h}
E. ritroides (Abali)	16.31 ± 0.01^{g}	9.05 ± 0.007^{b}	25.20 ± 0.007^{h}	53.88 ± 0.007^{b}
E. elbursensis (E-hashem)	9.62 ± 0.007^{a}	9.06 ± 0.007^{b}	18.19 ± 0.007^{a}	57.03 ± 0.007^{d}
E. robustus (Qom)	12.56 ± 0.007^{c}	12.43 ± 0.007^{g}	25.29 ± 0.007^{i}	52.73 ± 0.007^{a}
E. leiopolycerus (Kamard)	12.22 ± 0.007^{b}	$11.46 \pm 0.007^{c,d}$	23.54 ± 0.007^{f}	85.78 ± 0.007^{f}
E. leiopolycerus (Abali)	12.70 ± 0.007^{d}	11.41 ± 0.007^{c}	23.10 ± 0.007^{e}	82.96 ± 0.007^{i}

chromosome number was reported as $2n = 32$ (Ghaffari, 1999; Sheidai, 2000). However, recent work of Alijanpoor *et al*. (2019*b*) did not support the previous results, pointing to $2n = 32$ for *E. leiopolyceras* and $2n = 34$ for *E. ritrodes*. These contradictions might be explained by aneuploidy, dysploidy or unstable chromosome number in *Echinops* species.

In two meiotic cells of *E. ritroides* a ring quadrivalent was observed, but it was not considered in the final analysis due to its low frequency. Such quadrivalents may be formed due to heterozygote translocations among two pairs of chromosomes (Sheidai *et al*., 2010), conversely many univalent cases were observed in *E. elbursensis*. Dendrograms (Figs. 1 and 2) produced similar results which confirm previous reports (Rechinger, 1979; Mozaffarian 2008*b*) pointing toward the show close affinity between *E. chorassanicus* population and *E. leiopolyceras* population. *E. robustus* and *E. ritrodes* species are

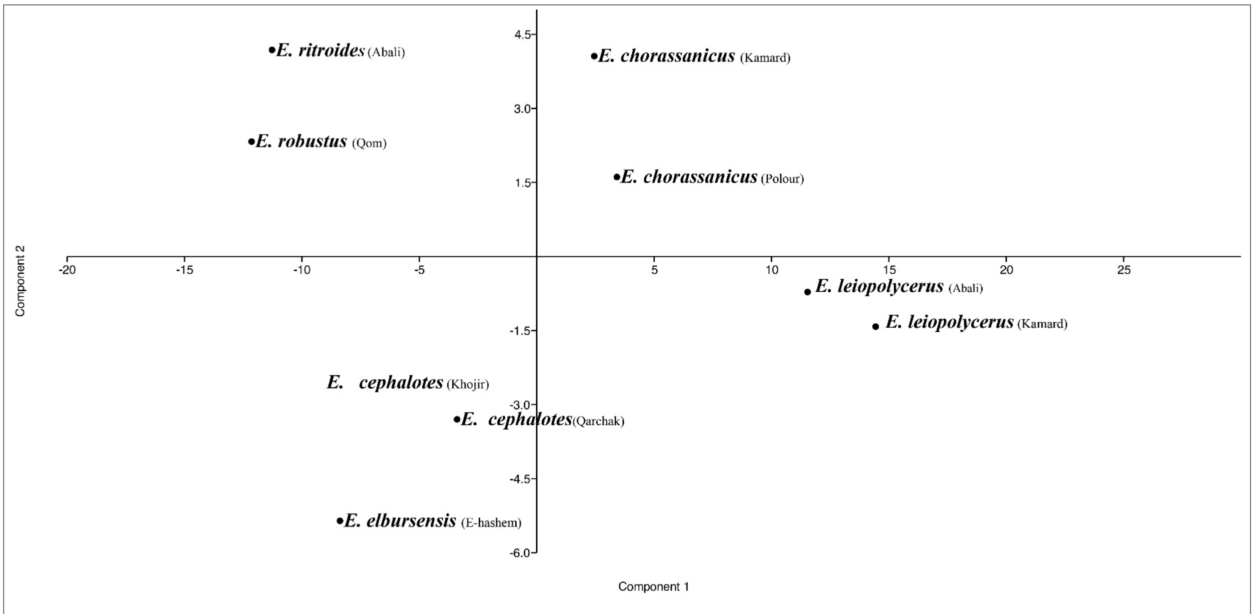

Figure 1. PCA plot analysis of meiotic data in the *Echinops* species and populations studied. The first component includes *E. cephalotes* (Khojir), *E. cephalotes* (Qarchak), and *E. elbursensis* (E-hashem). Other species such as *E. robustus* (Qom), *E. ritroides* (Abali), *E. chorassanicus* (Kamard), *E. chorassanicus* (Polour), *E. leiopolycerus* (Kamard) and *E. leiopolycerus* (Abali) belong to the second component.

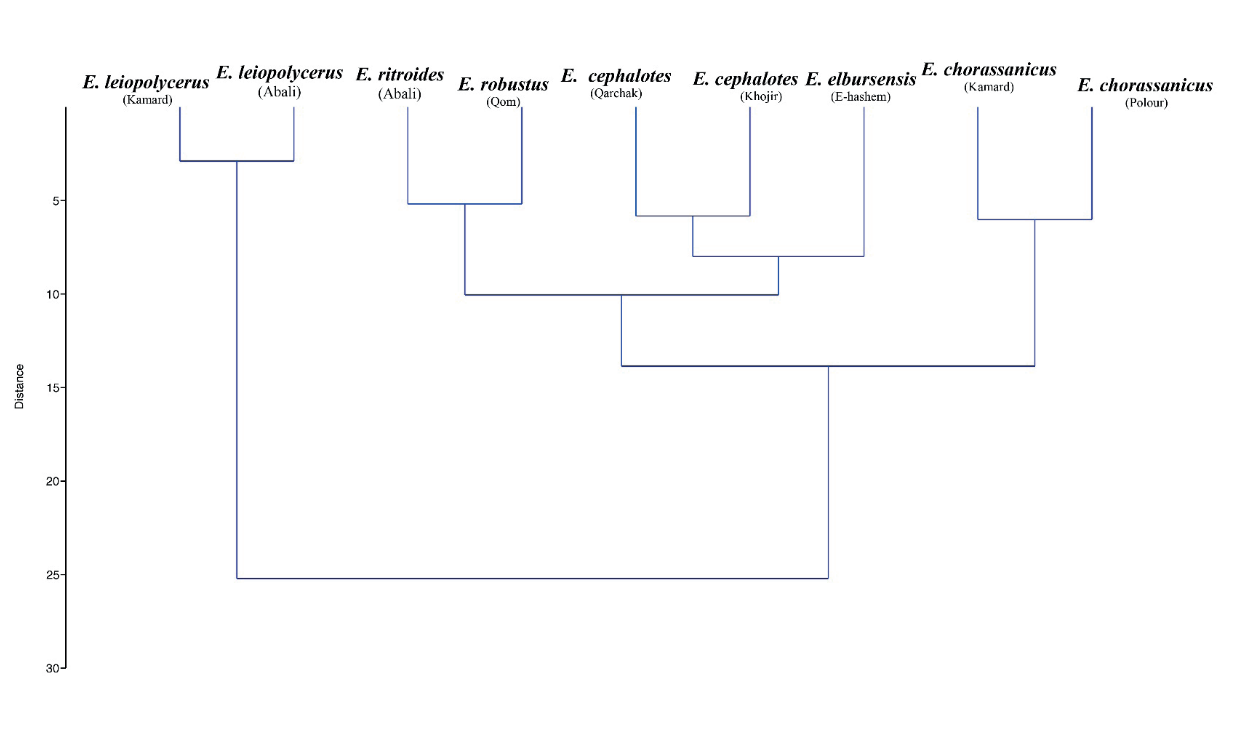

Figure 2. Cluster analysis (single linkage) of meiotic characters in *Echinops* species studied.

placed very close in the same clade. Comparatively, *E. elbursensis* remain closely related to *E. cephalotes* populations (Figs. 2 and 3). Chromosomal stickiness occurred in the early stages of prophase to the end of the meiotic stage in most of the studied species. Except for *E. elbursensis*, other species had unreduced pollen grains. However, in *E. retroides* its percentage was high (Table 2).

Some meiotic irregularities include laggard, anaphase, telophase II failures, stickiness, tripolar, and micronucleus, resulting in the formation of tripolar cells variation in pollen grain size with unreduced pollen grain (Bahattacharya, 1978; Sheidai *et al.*, 2010; Alijanpoor & Safaeishakib, 2022). In *E. ritroides* ($2n = 2x = 34$) from the Abali population, about fifty percent of unreduced pollen grains were obtained. However, in this species some $2n = 32$ cells were observed in meiosis. The main reason for this phenomenon is the presence of abnormalities, especially quadrivalents. On the other hand, there is a high level of stickiness observed in this species. Therefore, unreduced pollen grains with a variety of shapes (oval and triangular) in the studied species have been shown here [Fig. 3 (20)]. Moreover, tripolar and multipolar cell formations as meiotic irregularities can be due to anaphase I and II abnormality (Sheidai & Bagheri-Shabestarei, 2007) [Fig. 3 (18, 22)]. Meiotic cells with double chromosome number of chromosomes may be due to syncyte formation and lack of anaphase separation. Moreover, in accordance with some reports, phenomena like cytomixis (the migration of the nuclei from one cell to another through special intercellular channels), anaphase failure and multipolar cell formation could be responsible for unreduced gamete formation in some plant species (Sheidai & Fadaei, 2005; Sheidai & Bagheri-Shabestarei, 2007). Abnormal meiosis behavior seems to be one of the reasons for infertility in pollen grains.

CONCLUSIONS

This accurate cytological study of *Echinops* species displayed that chromosome stickiness and anaphase failure, leading to meiocytes with double chromosome numbers and multipolar cells, might be considered as the possible mechanisms of the unreduced pollen grain shape.

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